

# Comparison of two spot concentrated Sputum AFB Smear versus two direct Sputum AFB Smears done regularly in Revised National Tuberculosis Control Programme

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## ABSTRACT

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**Background:** Direct smear microscopy is one of the most reliable diagnostic methods for Pulmonary tuberculosis. The standard protocol involves 3 patient visits till he collects the result. Drop-out rates during this process are as high as 37%, due to multiple patient visits. Operational research is now focused on reducing the burden of smear microscopy without compromising its effectiveness as a case finding strategy. The diagnostic process could be made much more efficient and convenient for patients if it could be completed in a single day by examining 2 sputum specimens on the same day. Public health point of view also it would create a major impact, as it would help in curtailing transmission of the disease by earlier diagnosis.

**Materials and Methods:** All 1050 patients were clinically examined and then subjected to chest skiagram, complete blood count, renal and liver function tests, Mantoux and icc on the first day. The patients were advised to give good quality spot sputum of about 5ml, which will be divided into 2 equal parts. One was subjected to concentration and stained by Ziehl Nielson technique. The other part was smeared as per the regular RNTCP technique. Then after 1 hour another sample of 2-3 ml was again concentrated and smeared. The next day 2nd early morning sample was smeared under the RNTCP technique.

**Results:** There were 5 initial defaulters in the study. Out of them one was sputum positive. 2 HIV cases were present in the study. In them the detection rate was high in concentration method. There were 1050 smears done by RNTCP method and 1050 smears done by concentration technique that is a total of 2100 smears were analysed. Sample A - RNTCP 1<sup>st</sup> sample vs 1<sup>st</sup> spot concentrated sample. Sample B - RNTCP home sample vs 2<sup>nd</sup> spot concentrated sample. With regard to sample A, the proportion of positives detected by concentration method (27.4%) was significantly higher (mc nemar's test: p value= 0.0003) than that detected by the conventional method (24.1%). With regard to sample B, the proportion of positives detected by both methods were not significantly different (mc nemar's test: p value=0.06).

**Keywords:** Concentrated Sputum AFB smear, Spot Sputum AFB smears, Burden

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## INTRODUCTION

Direct smear microscopy is one of the most reliable diagnostic methods for Pulmonary tuberculosis. The standard protocol involves 3 patient visits till he collects the result. Drop-out rates during this process are as high as 37%, due to multiple patient visits. Operational research is now focused on reducing the burden of smear microscopy without compromising its effectiveness as a case finding strategy. The diagnostic process could be made much more efficient and convenient for patients if it could be completed in a single day by examining 2 sputum specimens on the same day. Public health point of view also it would create a major impact, as it would help in curtailing transmission of the disease by earlier diagnosis.

## AIM & OBJECTIVES

To evaluate the performance of 2 concentrated AFB smears obtained on a single day against 2 direct smears obtained over 2 days, in the diagnosis of Pulmonary Tuberculosis.

## MATERIALS AND METHODS

- Study design : Prospective Observational Study.
- Study Centre : Institute of Thoracic Medicine & Madras Medical College, Chennai
- Study period : February 2013 - February 2014
- Sampling : Simple random sampling
- Sample size : 1050

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f. Selection of Samples : Pulmonary Tuberculosis suspects

g. Definitions, procedure:

A pulmonary TB suspect is defined as:

- An individual having cough of 2 weeks or more
- Contacts of smear-positive TB patients having cough of any duration
- Suspected/confirmed extra-pulmonary TB having cough of any duration
- HIV positive patient having cough of any duration

### Inclusion Criteria

- Individuals with age greater than 12yrs age
- An individual having cough of 2 weeks or more
- Contacts of smear-positive TB patients having cough of any duration
- Suspected/confirmed extra-pulmonary TB having cough of any duration
- HIV positive patient having cough of any duration

### Exclusion Criteria

- Individuals with hemoptysis
- Individuals who are not able to bring out sputum

### PROCEDURE

All consenting participants would be clinically examined and then subjected to chest skiagram, complete blood count, renal and liver function tests, Mantoux and icc on the first day. The patients would be advised to give good quality spot sputum of about 5 ml, which will be divided into 2 equal parts. One would be subjected to concentration and stained by Ziehl Nielson technique. The other part would be smeared as per the regular RNTCP technique, then after 1 hour another sample of 2-3 ml will be again concentrated and smeared. The next day 2nd early morning sample would be smeared under the RNTCP technique.

### Sample processing

Concentration technique - Equal amount of sputum (3ml) and phenol ammonium Sulphate solution was mixed and shaken in a plastic container till the mucus dissolves. The sample was then centrifuged; the sediment was carefully isolated and smeared. It was then stained using Ziehl Nielsen technique and examined under oil immersion microscopy. RNTCP smears and concentrated smears were read by 2 separate technicians and

were double and cross blinded.

### RESULTS & ANALYSIS

There were 5 initial defaulters in the study. Out of them one was sputum positive. 2 HIV cases were present in the study. In them the detection rate was high in concentration method. There were 1050 smears done by RNTCP method and 1050 smears done by concentration technique that is a total of 2100 smears were analysed. Sample A - RNTCP 1<sup>st</sup> sample vs 1<sup>st</sup> spot concentrated sample. Sample B - RNTCP home sample vs 2<sup>nd</sup> spot concentrated sample. With regard to sample A, the proportion of positives detected by concentration method (27.4%) was significantly higher (mc nemar's test: p value= 0.0003) than that detected by the conventional method (24.1%). With regard to sample B, the proportion of positives detected by both methods was not significantly different (mc nemar's test: p value=0.06).

### LIMITATIONS

The results of the smears were not compared with the culture.

### CONCLUSIONS

- 2 spot concentrated smears itself is as good as 2 direct smears done by RNTCP method.
- Less number of patient visits
- Less number of drop outs in the diagnostic pathway.
- Less cost to the patient and cost effective to the programme.
- Early diagnosis and initiation of treatment

The performance of concentrated smears in field conditions should be evaluated further.

### END NOTE

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**Conflict of Interest:** None declared

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## REFERENCES

1. Verma SK, Mahajan V. HIV-Tuberculosis Co-Infection. *Int J Pulmonary Med* 2008; 10 :( 1)
2. RNTCP- An overview. A manual for sensitization of Medical College Faculty. 1<sup>st</sup> Ed. Mumbai: Maharashtra State TB Control Society; Jul 2004.
3. Kant L. Improving detection of infectious cases. *Indian J Tuberc* 2000; 48:115-6.
4. Sarin R, Mukherjee S, Singla N, Sharma PP. Diagnosis of Tuberculosis under RNTCP: Examination of two or three sputum specimens. *Indian J Tuberc* 2001; 48:13-6.
5. Giacomelli LRB, Helbel C, Ogassawara RLN, Barreto AMW, Martins FM, Cardoso CL, et al. Improved laboratory safety by decontamination of unstained sputum smears for acid-fast microscopy. *J Clin Microbiol*. 2005 Aug;43(8):4245–8.
6. Miörner H, Ganlöv G, Yohannes Z, Adane Y. Improved sensitivity of direct microscopy for acid-fast bacilli: sedimentation as an alternative to centrifugation for concentration of tubercle bacilli. *J Clin Microbiol*. 1996 Dec;34(12):3206–7.
7. Makunde WH, Makunde RA, Kamugisha LM, Mgeme SG, Liwa A. Improved microscopy diagnosis of pulmonary tuberculosis using sodium hypochlorite concentration technique in Tanga, Tanzania. *Tanzan Health Res Bull*. 2007 May;9(2):87–93.